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1162-Plat**Roadblocks on the E.Coli Genome: The Workings of a Molecular Mouse Trap at the Single-Molecule Level**

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During DNA replication in E.coli, replication forks are prevented from moving beyond the termination site by Tus proteins that bind to specific, asymmetric Ter DNA sites. The Tus-induced blockage is known to depend on the fork's direction of approach. It is hypothesized that strand separation on the blocking side triggers the formation of a molecular roadblock by the binding of a base to a high affinity site on the Tus protein. In this study, we use single-molecule multiplexed magnetic tweezers to investigate the origin of the asymmetry of the Tus-Ter block. We use 1kb DNA hairpins that contain a single Ter site in either the permissive or non-permissive orientation. Application of pulling forces >16pN causes mechanical unzipping of the hairpins, thereby mimicking the DNA unwinding that accompanies DNA replication. When wtTus binds to the hairpin with the permissively oriented Ter site, strand separation is transiently blocked; conversely, when wtTus is bound to the non-permissive Ter hairpin, strand separation is fully blocked. Under physiological conditions the non-permissive Tus-Ter lock was ubiquitous, independent of the loading rate, and withstood the largest forces we could apply (>70pN). Interestingly, the probability of lock formation strongly depends on the ionic strength of the solution. Our ability to distinguish simple DNA binding from the locking process and to apply this to a series of Tus mutants allows us to put together a complete picture of the Tus-Ter lock.